Improving the Anti-Cancer Effect Of T-Cells by T-Cell Receptor Engineering

November 19, 2012

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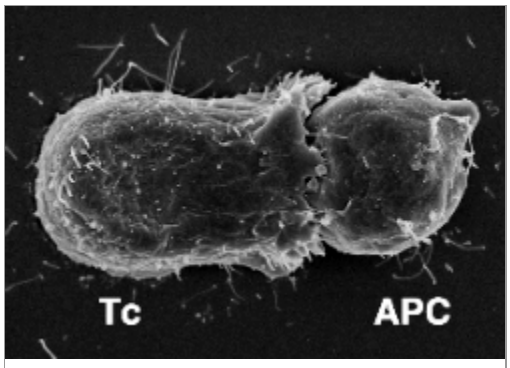
There are two populations of T-cells that are characterized by the type of T-cell receptor (TCR) that is expressed: αß and γδ. The majority of T-cells express αß TCRs while only 2% of total T-cells have TCRs made of γδ subunits. Furthermore, a specific type of γδ T-cell that expresses γ9δ2TCRs, a class of innate immune cells, can be highly anti-tumorgenic in mouse cancer models. However, adoptive transfer of γ9δ2 cells in the clinic has provided less tumor control. The Strong (Basic Sciences Division) and Kuball (UMC Utrecht, The Netherlands) labs investigated two important questions: what is the molecular defect curtailing the anti-tumor effectiveness of γ9δ2T-cell therapy and can this weakness be ameliorated to increase their therapeutic potential?

As a starting point, γ9δ2T-cells from a healthy donor were cloned and their reactivity against a panel of tumor cells was assessed by an IFNγ ELISA assay. Individual clones of γ9δ2T-cells displayed differential recognition of and activation by specific tumor cells. They hypothesized that the strength of the anti-tumor response correlated with differences in γ9δ2TCR amino-acid sequence. To test this, the γδ chains of a T-cell clone with strong functional avidity was sequenced and compared to a T-cell clone that displayed a weak response in the ELISA assay. Interestingly, only the CDR3 domain of the receptors contained amino acid changes. To determine if the amino acid changes influenced TCR function, the mutant receptors that displayed increased functionality and wild-type control receptors were transduced into peripheral blood αßT-cells and T-cell activity against tumor cells was determined. Indeed, αßT-cells expressing the stronger γ9δ2TCR had a greater ability to lyse tumor cells and secrete IFNγ compared to wild-type γ9δ2TCR-expressing αßT-cells. Thus, the CDR3 domain of the γ9δ2TCR is a critical determinant of TCR functionality.

Mutational analysis of CDR3 domain residues in both the γ9 and δ2 chains of wild-type receptors revealed that too short (no amino acid addition) and very long (12 amino acid) alanine stretches in a particular CDR3 region negatively affect receptor function. To determine if an optimal CDR3 length exists, they searched the ImMunoGeneTics database for stretches of amino acids in the CDR3 regions of the γ9δ2TCRs. The majority of listed γ9δ2TCRs contained a conservative number of residues (5-7 amino acids) in the CDR3 domain, indicating that those TCRs have a functional advantage and are under positive selection. In addition, single point mutations in both chains of the

γ9δ2TCRs were found to influence TCR functional avidity. Lastly, CTE (combinatorial-γδTCR-chain-exchange) was used to engineer γ9δ2TCRs that were predicted to have increased receptor function based on the aforementioned results. Astoundingly, αßT-cells expressing the CTE-engineered γ9δ2TCRs were highly reactive against a broad range of tumors and did not affect normal tissue in the IFNγ ELISA. When tested in a humanized mouse model, the CTE-engineered γ9δTCRs decreased tumor outgrowth and increased the overall survival of the mice. Taken together, these data indicate that CTE-engineered TCRs are a promising candidate for clinical application and may prove useful for future anti-cancer therapy.

Gründer C, van Dorp S, Hol S, Drent E, Straetemans T, Heijhuurs S, Scholten K, Scheper W, Sebestyen Z, Martens A, Strong R, Kuball J. 2012. γ9 and δ2CDR3 domains regulate functional avidity of T-cells harboring γ9δ2T-cell receptors. *Blood*. Epub ahead of print, doi: 10.1182/blood-2012-05-432427.



Obtained from Institut Pasteur website.

A scanning electron micrograph of a T lymphocyte (Tc) recognizing and attaching to an antigen presenting cell (APC).